Journal of Forensic and Legal Medicine 18 (2011) 34–37

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Case report

Forensic DNA expertise of incest in early period of pregnancy

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ARTICLE INFO

Article history:
Received 23 May 2010
Received in revised form
29 September 2010
Accepted 24 November 2010
Available online 13 December 2010

Keywords:
Incest
DNA
Prenatal
Histology

ABSTRACT

Proving incest from tissue obtained by abortion early in pregnancy can be a challenge. Problems include the small quantity of embryonic tissue in the products of conception, and the mixing of DNA from mother and embryo. In many cases, this amorphous material cannot be grossly segregated into maternal and fetal components. Thus, morphological discrimination requires microscopy to select relevant tissue particles from which DNA can be typed. This combination of methods is reliable and efficient. In this article, we present two cases of incest discovered by examination of products of conception.

1. Introduction

Adolescence is a sensitive period of physical, sexual and psychosocial development. Each teenager becomes an individual with a unique personality.[1] Most commonly, incest is defined as mating between first-degree relatives, i.e., father–daughter, mother–son, or brother–sister, who have 30–50 percent of their genes in common. However, in some countries the definition may be widened to include half-sib and uncle–niece unions.[1–4] The dynamics of the brother–sister incest relationship have received far less attention than the father–daughter affair. Assumptions abound but, there has been a conspicuous lack of documentation despite estimates that incest between siblings may be five times more common than paternal incest. Sibling incest is more likely to occur if there is parental neglect or abandonment, as brothers and sisters turn to each other for comfort, nurturance and identity, or as a means of expressing rage and hurt. Also, this may appear through exposure to pornography or sexually explicit material, sex talked about openly in obscene words, and where parents make little effort to prevent children from seeing them engage in marital or extramarital activity in the home.[5] Legally, incest and sexual aggression toward minors are classified as a criminal behavior even if the perpetrators are minors themselves. Incest has psychological, social, medical, and legal ramifications.[6]

In some rape cases, sperm is not available from vaginal swabs and the only resulting genetic evidence can be products of conception. By determining parentage, it is possible to link the act to the perpetrator. In fact, genes from the suspect become part of the genetic makeup of the products of conception.[7] In the event of pregnancy after rape, paternity determination to identify the perpetrator can be performed prenatally by biopsy, by amniocentesis or by using abortion material as the source of DNA.[8] After termination of pregnancy, the abortion material consists of a mixture of fetal and maternal tissue and blood. Typing of short tandem repeat (STR) loci from fetal components has been very effective, but complications may arise if maternal tissue predominates, wherein the interpretation of the results may be more complicated. In many cases, this amorphous material cannot be segregated into maternal and fetal components upon gross dissection. Thus, morphologic discrimination frequently requires microscopy, and relevant tissue particles can be selected after histological examination. This combination of methods, not uncommon in clinical medicine, increases the reliability of the results.[9,10] In similar situations, biopsy of chorionic villi has been used as a tool for forensic paternity testing.[8,9,11]

We present two cases of incest that were investigated in the early period of pregnancy.

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doi:10.1016/j.jflm.2010.11.012

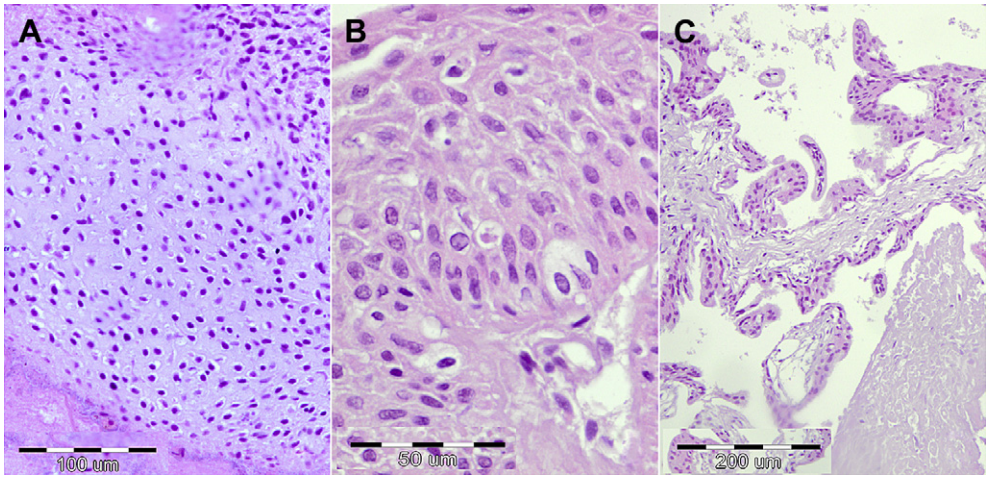

Fig. 1. Tissue analyzed from case 2. Histological appearance of cartilage (A), epidermis (B), and chorionic villus (C). Hematoxylin and eosin. Scale bar: 50 microns in A, 100 microns in B, 200 microns in C.

2. Case report

2.1. Case 1

In the first case, there was incest between brother and sister. Labour was induced with prostaglandin during the 15th week of pregnancy. The victim, a 14-year-old girl, stated before the investigative authorities that she had been raped by a 60-year-old man. Forensic DNA analysis of paternity included blood of the victim, blood taken from the heart of the fetus and blood of the suspect. Later in the forensic examination blood from her father and brother was analyzed.

The isolation of DNA from the blood of the victim, the fetus, the suspect, the father and the brother of the victim was performed with a Qiagen Mini kit.

2.2. Case 2

The second case was incest between uncle and niece. Vacuum abortion was performed in the seventh week of pregnancy. According to the information obtained from the investigating authorities, the 14-year-old victim had been raped by her uncle. For the purposes of forensic DNA analysis, we were provided with material from the performed abortion, blood of the victim and blood of the suspect.

Since it was impossible to identify which parts belong to the fetus in the amorphous mass obtained from the abortion by gross examination, the entire mass was fixed in 10% neutral formalin, and embedded in 14 paraffin blocks, in order to determine the presence of fetal tissue by histology. The blocks were cut into 5 micron-thick serial slices in order to be able to identify embryonic tissue deep within the blocks. The slices were stained with hematoxylin–eosin. Embryonic tissue was found in 3 paraffin blocks, and 1 contained chorionic villi (Fig. 1).

Deparaffination of the embedded tissue was performed using the Qiagen mini kit protocol for deparaffinization. DNA was extracted from the fetal tissue and placenta in the paraffin blocks, by the phenol–chloroform method.[12]

3. STR typing

Multiplex PCR amplification was performed using 1–3 ng of genomic DNA according to the manufacturer's protocols for the AmpFISTR Identifiler kit and AmpFISTR YFiler kit. Amplification was carried out in a 9600 Thermal Cycler (Applied Biosystems). For electrophoresis 1.5 μl of the PCR product was combined with 12 μl of formamide and 0.5 μl of GeneScan 500 LIZ size standard. The detection of PCR products and genotyping were carried out on an ABI PRISM 310 Genetic Analyser (Applied Biosystems) using the ABI PRISM collection software, ABI Prism 310 Data Collection software

Table 1
Profile analysis results for autosomal STR's – Case 1 (excluding loci underlined).

	Locus	Chromosomal location	Victim	Embryo	Suspect	Father	Brother
1	D8S1179	8	13/13	13/13	12/13	13	13
2	D21S11	21q11.2-q21	29/32.2	29/32.2	27/28	32.2/33.2	29/32.2
3	D7S820	7q11.21-22	10/11	10/11	8/11	11	10/11
4	CSF1PO	5q3.3-34	10/11	10/11	10/11	11	10/11
5	D3S1358	3p	15/18	15/16	15/16	16/18	15/16
6	TH01	11p15.5	7/9	9/9	9.3/9.3	7/9.3	7/9
7	D13S317	13q22-31	8/10	8/11	11/11	8/10	8/11
8	D16S539	16q24-qter	12/12	10/12	12/13	10/12	10/11
9	D2S1338	2q35-37.1	22/25	22/25	24/25	22/25	25
10	D19S433	19q12-13.1	13/13	13/15.2	14/14	13/15.2	13/15.2
11	vWA	12p12-pter	18/18	17/18	16/18	18	17/18
12	TPOX	2p23-pter	8/8	8/8	8/11	8/11	8
13	D18S51	18q21.3	15/21	14/21	14/18	14/21	14/15
14	D5S818	5q21-31	11/13	13/13	12/12	13/14	11/13
15	FGA	4q28	21/22	21/22	21/21	22/24	22
16	Amelogenin	X; p22.1-22.3 Y:p11.2	X/X	X/X	X/Y	X/Y	X/Y

3.1.0 and Gene Mapper v 3.2 (Applied Biosystems). The statistical analyses were conducted using DNA View software based on a study of the Macedonian population for autosomal STR's.[13]

Statistical analyses were conducted using DNA View software version 28.46.

4. Results

4.1. Case1

The results obtained in the profile analysis of the autosomal STR's of the victim, the fetus and the suspect indicated that there was no match at 6 loci with the paternal alleles, ruling out the suspect as a possible biological parent. In the analysis of the alleles of the victim and the fetus it was determined that the victim and the fetus shared 23 of 30 possible alleles (77%), raising suspicions of incest (Table 1).[14] In the subsequent analysis involving the victim's father and brother, the brother could not be excluded from paternity. The observed genetic results are more than one million times more likely if the victim's brother was also the father of the child, than an unrelated man being the father.

4.2. Case 2

The results obtained in the profile analysis of the autosomal STR and Y chromosome, the STR of the victim, the embryo and the suspect, supported the suspicion that the uncle was the embryo's father with a likelihood greater than 100,000 (Tables 2 and 3).

5. Discussion

There are several reports on the use of DNA probes on fetal tissue and mixtures of placental and fetal cells in paternity cases.[10] Many technical problems may be encountered. Sample size, tissue type, and time elapsed after abortion are critical to the success or failure of the method. The type and the size of the fetal samples depend on the abortion method.[7] According to some authors, the optimal sample consists of fetal blood obtained by heart puncture, which is only possible in older fetuses.[15]

In the first case, the abortion was performed at the beginning of the second trimester of pregnancy, when the fetus had already been formed. The isolation of DNA from the fetus posed no problem whatsoever, since blood was taken from the heart of the fetus, and there was no possibility of contamination by the mother's blood. We showed the impossibility of the suspect's paternity, and he was released. We also established the existence of brother–sister incest.

Table 2
Profile analysis results for autosomal STR's – Case 2.

	Locus	Chromosomal location	Victim	Embryo	Suspect
1	D8S1179	8	13/14	13/14	10/13
2	D21S11	21q11.2-q21	30.2/32.2	30.2/31.2	30.2/31.2
3	D7S820	7q11.21-22	9/12		10/11
4	CSF1PO	5q3.3-34	11/12	11/12	11/12
5	D3S1358	3p	18/18	17/18	17/18
6	TH01	11p15.5	9/9.3	6/9.3	6/9.3
7	D13S317	13q22-31	9/12	8/9	8/12
8	D16S539	16q24-qter	8/10	8/11	11/12
9	D2S1338	2q35-37.1	20/23	23/23	23/23
10	D19S433	19q12-13.1	13/15	13/15	13/15
11	vWA	12p12-pter	15/17	15/17	17/18
12	TPOX	2p23-pter	9/11	8/11	8/8
13	D18S51	18q21.3	12/16	12/16	12/17
14	D5S818	5q21-31	12/12	10/12	10/11
15	FGA	4q28	21/24	21/24	21/25
16	Amelogenin	X; p22.1-22.3 Y:p11.2	X/X	X/Y	X/Y

Table 3
Profile analysis results for Y-chromosomal STR's – Case 2.

	Locus	Embryo	Suspect
1	DYS456	15	15
2	DYS389I	14	14
3	DYS390	22	22
4	DYS389II	30	30
5	DYS458	17	17
6	DYS19	15	15
7	DYS385a/b	15/17	15/17
8	DYS393	12	12
9	DYS391	10	10
10	DYS439	11	11
11	DYS635	20	20
12	DYS392		11
13	Y GATA H4	13	13
14	DYS437	14	14
15	DYS438	9	9
16	DYS448	19	19

The victim's deliberate false statement before the investigative authorities was intended to protect her brother.

In the second case, the abortion was performed by suction in the early stage of the first trimester. As fetal tissue could not be identified by eye, fetal and placental material was isolated from paraffin blocks after histology. This method has proven to be simple, rapid, inexpensive and effective. Similar methods have been described in the available literature referring to determination of the cells of fetal tissue or DNA isolation from chorionic villi.[7–11,16,17]

In the majority of the cases of rape or incest the victim decides to have an abortion in order to restore her psychological and physical health.[18] In most rape or incest cases, the victim suffers severe psychological consequences, such as posttraumatic stress syndrome, depression or frigidity, etc.[19–23] Certainly, also the close relatives, especially the incest victim's mother, also suffer stress.[24] Furthermore, fear of recessive abnormality is also a reason for an abortion.[25]

The question arises of whether to wait for the incest victim's pregnancy to enter a more advanced stage of the first trimester of pregnancy, when the fetus is larger and it is easier to identify the fetal material for the DNA analysis, which would facilitate the forensic examination or, on the other hand, to perform an abortion as soon as possible.

Incest victims, who in most cases are children or teenagers, are rapidly changing physically and psychologically and may suffer serious consequences in their further development.[26] Therefore, if an abortion is to be performed it is be best to be done in the early stage of the first trimester. At the same time, the victim and her family should be referred to therapy.[27]

Conflict of interest
None declared.

Funding
None.

Ethical approval
None declared.

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